Comparison of *Klebsiella pneumoniae* from mastitic and normal swine and their environment

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INTRODUCTION

Postparturient disorders of the sow causing lactation failure, such as the agalactia syndrome, have increased in importance along with the increase in intensive pork production in many areas of the world. Although these disorders appear to be etiologically complex, one rather common feature is infection of the mammary gland caused by coliform bacteria such as Klebsiella pneumoniae and Escherichia coli. Klebsiella mastitis often is striking in its severity. The condition can occur as a separate entity, or as part of the agalactia syndrome. In either case, it may have serious economic consequences. The loss of baby pigs due to decreased milk supply is often extensive, and many sows in a herd may be involved. In contrast to other diseases associated with lactation failure, it is not unusual for the sow to die from Klebsiella mastitis unless treated promptly.

Information concerning Klebsiella mastitis in the literature has been mainly limited to reports of individual cases or herd outbreaks. Much of the knowledge available has been from investigations of coliform mastitis in cows. Several studies have been undertaken recently, however, to clarify the epizootiology and pathogenetic mechanisms in coliform mastitis in the bovine and in swine, and some effort has been made to define the role that organisms from the environment play in causing the disease.

The present investigation was undertaken to compare strains of Klebsiella pneumoniae isolated from mastitic swine, normal swine, and the swine environment. An attempt was made 1) to find any trends suggesting that strains with certain morphologic, biochemical, or serologic

characteristics were associated with swine mastitis; 2) to find *in vitro* methods to evaluate the pathogenicity of *Klebsiella* strains; and 3) to compare the virulence of *Klebsiella* strains from different sources by intramammary inoculation of sows.

LITERATURE REVIEW

Coliform Mastitis

Swine mastitis

Mastitis is a significant disease of swine, causing extensive loss of pigs and occasionally the death of the sow. The condition can also be seen as part of the agalactia syndrome, which is an acute disease of sows characterized by complete or partial failure of milk secretion. This syndrome is often referred to by other names, including mastitis-metritis-agalactia syndrome (C. Martin et al., 1967), agalactia toxaemica (Ringarp, 1960), and agalactia (Armstrong et al., 1968; Swarbrick, 1968).

Several workers have described organisms of the Klebsiella group as the cause of acute mastitis in swine (Adler, 1951; Helmboldt et al., 1953; Langham and Stockton, 1953; Easterbrooks and Plastridge, 1956; Jamkhedkar et al., 1964; Lake and J. Jones, 1970). The disease usually occurs within 36 hours of parturition. Pyrexia (41-42°C), anorexia, tachypnea, coprostasis, and purple discoloration of the skin are common symptoms. A varying number of glands may be affected, being hard and swollen, with their secretion being viscous or containing clots. The sow may be reluctant to stand, have a hoarse voice, and occasionally have a serous or mucoid discharge from the vulva. In fatal cases, the picture is one of acute toxemia.

Lake and J. Jones (1970) reported that acute mastitis could be reproduced by the inoculation of a Klebsiella culture into the mammary glands of recently parturient sows.

Lake (1972) speculated on the pathogenesis of Klebsiella mastitis, stating that a sow's udder is more exposed to fecal contamination than is a cow's, and that several glands may become affected simultaneously. However, the large number of involved glands and generalized infection in the sow indicate a possible hematogenous infection that may originate from another site, e.g., a single mammary gland or the uterus. Different capsular serotypes of Klebsiella have been isolated from the same herd and even the same sow (Lake and J. Jones, 1970). Lake points out, however, that it is possible to isolate the same capsule type from several mammary glands of an affected sow, from successive cases of mastitis in a herd, and from the prepuce of boars in the herd. Klebsiella spp. can also be readily isolated from udder surfaces, nasal secretions, feces, pen floors, bedding, feeding troughs, water bowls, and in large numbers from the rectal contents of pigs of affected sows (Nieva, 1971; Lake, 1972). The piglets may not suffer illness, but may act as disseminators of the organism. Lake suggested that conditions be provided in the farrowing house that would minimize survival of coliform bacteria.

Maclean and Thomas (1974) examined the bacterial flora of the feces and vaginal secretions of sows during the reproductive cycle. They found a significant increase in potentially pathogenic Escherichia coli on one farm associated with parturition and dietary change, and a similar increase in Klebsiella spp. on another. In both cases, the increase in coliforms correlated well with a high incidence of disease caused by the group of organisms with the higher excretion rate. Isolation of Klebsiella from the vagina and rectum went from zero at mid-pregnancy to 40%

and 27% respectively on the first day after farrowing. They proposed that stress from parturition may predispose to increased excretion of potential pathogens.

Numerous cases of mastitis and agalactia associated with E. coli have been described (Venn, 1941; Jackson, 1952; Hebeler, 1954; Sumner, 1957; Brooksbank, 1958; Noble et al., 1960).

H. Smith (1965) felt that the isolation of pathogenic E. coli from swine was increasing, possibly due to the wide use of antibiotics in feed. He observed that many different serotypes are involved in the syndrome, and speculated that certain strains may become extremely virulent and even undergo mutation. He also speculated that clinically normal swine may be carriers of pathogenic E. coli.

Ringarp (1960) and Berner and Marx (1967) stated that there apparently is no bacterial infection in healthy swine mammary glands. T. McDonald and J. McDonald (1975), however, reported 30.2% of the glands of 24 necropsied sows were infected with aerobic bacteria just before farrowing. They defined an infected gland as one containing 200 or more organisms of similar characteristics per milliliter of milk. They observed that cases of agalactia commonly occur throughout parity groups and speculated that this may be related to common environmental exposure to bacteria that cause mastitis.

Ringarp (1960) provided the first thorough investigation of the agalactia syndrome in sows. He studied 1180 cases and classified 88.6% of them as agalactia toxaemica. This condition was described as decreased milk secretion occurring 12 to 48 hours after farrowing, accompanied by malaise, anorexia and coprostasis. Udder swelling usually started in the

hind sections and proceeded forward, though it could be more obvious in one or several sections. Occasional symptoms were pyrexia, increased lochial discharge, stiffness, mental disturbance, and mottled skin. He was able to reproduce the symptoms of agalactia toxaemica by making drastic feeding changes immediately after parturition, but not 5 to 12 days later. He concluded from this that the stress and hormonal disturbances caused by parturition create conditions suitable for the occurrence of the disease symptoms. He further suggested that selection for rapid growth rate in pigs may have involved selection of animals with abnormal endocrine function, and that such animals may be more prone to develop endocrinologic dysfunction during lactation. He found no reason to believe that agalactia toxaemica infection had a galactogenic origin, since the udder lesions usually came quickly and simultaneously in most glands. He viewed his experimental production of the syndrome as proof that changes in the intestine and its bacteriological flora were of primary aetiological significance. Coliform bacteria dominated the bacterial flora of the udder and uterus in both spontaneous and experimental cases of agalactia toxaemica. Penny (1967) and Thurman and Simon (1970) also reported that E. coli was the most common organism isolated from sows with agalactia. Ringarp was able to prevent agalactia toxaemica with injections of polyvalent coliserum before farrowing. Other authors have recommended polyvalent E. coli antiserum for the prevention of agalactia and mastitis (Jackson, 1952; Sumner, 1957).

Berner and Marx (1967) demonstrated that an inflammatory response, evidenced by increased cell content, always accompanied the isolation of E. coli in pure culture or large numbers from sows' milk.

Armstrong et al. (1968) examined 60 strains of E. coli isolated from 18 sows with agalactia and found that nearly every one was serologically distinct. They felt that the failure to isolate a predominant species of bacteria from most of the sows indicated that the syndrome was not an infectious entity. They believed that the bacteria may be secondary invaders, and in that way contribute to the overall pathogenesis of the disease.

Kopf (1967) was able to reproduce the agalactia syndrome by inoculation of mucoid E. coli into the sow's vagina. He pointed out that the tissue resistance of the host and the virulence, number and toxicity of the bacteria may all be important in the production of the disease. There was some evidence pointing to the influence of the stress of large litters, hormone imbalance, and lack of prior exposure to the organism. His results also suggested that the mucoid property may be associated with the virulence and toxicity of E. coli.

Ross et al. (1969), however, were not able to produce clinical symptoms by intravaginal inoculation of E. coli, though they pointed out that their isolates were smooth rather than mucoid. They did find that the presence of E. coli in the vaginal exudate of a post-partum sow was associated with reduced pig livability and growth, but did not believe it to be a primary factor. They also reported that, of sows naturally infected with E. coli, virtually every one studied had a different type of the organism.

J. Jones (1971) pointed out that the mammary gland, which is the organ central to the whole problem of lactational failure, has received little critical attention. He suggested that the more obvious involvement

of only a few glands might indicate an inflammatory response to infection rather than a systemic dysfunction.

Nachreiner and Ginther (1969) found that, although mastitis was significantly associated with agalactia, the presence of vaginal discharge was not. In addition, their survey of 74 swine producers revealed that the frequency of the agalactia syndrome was positively related to the swine population or intensity of production on a farm. There also was a positive correlation between the frequency of antibiotic use and the incidence of the disease. The same authors (Nachreiner and Ginther, 1972a) reported that the percentage of glands with mastitis was significantly higher in sows with agalactia than in normal sows. Others have also found mastitis to be the most consistent sign in the agalactia syndrome (C. Martin, 1970). Nachreiner and Ginther (1972b) produced evidence again in 1972 that mastitis may play an important role in swine agalactia, and stated that the syndrome may involve exposure to adverse environmental conditions at a critical time for the sow's physiology. They induced mastitis by injecting killed Staphylococcus aureus into the teat canal. The effect of this induced mastitis was apparently not only local, since feed consumption, respiratory rate, recumbency, cervix weight, number of ovarian follicles less than 4 mm in diameter, plasma corticosteroid, and number of circulating basophils were all altered. In 1974, they determined that gilts injected intramammarily with E. coli endotoxin had the same changes as those suffering from agalactia. The gilts responded similarly whether the injections were given on the day of parturition or 7 days post-partum (Nachreiner and Ginther, 1974).

Bovine mastitis

Reports of Klebsiella mastitis in cows are more common than those concerning mastitis in swine (Gilruth and MacDonald, 1911; F. Jones, 1918; Gwatkin et al., 1938; Murphy and Hanson, 1943; Burkhardt et al., 1943; Buntain and Field, 1953; Barnes, 1954; Easterbrooks and Plastridge, 1956).

Gilruth and MacDonald (1911) and Schalm and Woods (1952) felt that the sporadic nature of the disease indicated the organisms were harmless environmental saprophytes that once in a while became pathogenic. Hinze (1956) reported that Klebsiella spp. were shed intermittently from normal cows and speculated that for some undetermined reason such strains occasionally became virulent enough to cause severe mastitis. White (1957) described cases of Klebsiella mastitis in cows and Klebsiella pneumonia in calves of the same herd. He stated that the organisms were transmitted to the calves by some means other than the milk, since not all of the calves were fed milk from infected cows.

J. McDonald and Packer (1968) demonstrated that a small percentage of teat-end exposures to K. pneumoniae and Streptococcus agalactiae resulted in udder infection, but that these organisms were no more invasive for the mammary gland than Staphylococcus epidermidis. They concluded that the degree of exposure was much more important than invasiveness in the development of coliform mastitis.

T. McDonald et al. (1970), in a study of Gram-negative rods from bovine udder infections, reported that 84.3% were isolated from rear quarters. They suggested that the discharges of the urogenital or digestive tract were probable sources of infecting organisms. Many different serotypes of E. coli were isolated, indicating that there was no single

highly invasive strain causing the high incidence of this type of mastitis. Barnes (1954), Easterbrooks and Plastridge (1956), and Braman et al. (1973) reported the isolation of various Klebsiella serotypes from mastitic herds, indicating that Klebsiella mastitis is not caused by a single serotype of the organism.

Nieva (1971) was able to isolate K. pneumoniae from bovine nasal secretions, udder skin, feces, drinking water, mangers, floors, and walls. Braman et al. (1973) demonstrated that K. pneumoniae was easily isolated from sawdust bedding and drinking water in herds with Klebsiella mastitis. Howell (1972) and Brander (1973) implicated dirty cubicles (stalls), sawdust bedding, and contaminated water supplies as sources of coliforms that may cause mastitis. Brander stated that the organisms don't readily survive drying and are therefore most likely found in wet, poorly drained areas. He felt that trends toward increased herd size and new management methods might be altering the bacterial flora to favor increased survival and growth of environmental Gram-negative organisms. He mentioned that outbreaks tend to occur at periods of high susceptibility, e.g., at calving.

A study by Newman and Kowalski (1973) implicated wood by-products as sources of potentially pathogenic Klebsiella spp. The organisms were isolated from sawdust in sawmills and from both fresh and used sawdust on a farm with an outbreak of Klebsiella mastitis. Klebsiella spp. were isolated from 54% of the cows on the first sampling. Thereafter, the bedding was changed to sand or straw, and the second collection yielded the organisms from only 9% of the cows. Not all of the Klebsiella positive animals had clinical or subclinical mastitis. This led the authors

to conclude that the presence of the bacteria doesn't necessarily lead to an inflammatory reaction in the mammary gland, and some other factor, such as trauma, may be needed to initiate mastitis.

Bramley (1974) pointed out that other mastitis organisms are transferred from cow to cow, while the primary source of coliform mastitis organisms is the environment. His findings indicated a direct relationship between the degree of exposure to coliforms from bedding and the coliform infection rate. The incidence of coliform infection was highest in the hind quarters, and he believed that this may have been because they were more often in contact with the bedding. Sawdust favored the multiplication of Klebsiella spp., especially at the higher temperatures reached when it is used as bedding, but the organisms were also isolated in small numbers from unused sawdust. Change of bedding from sawdust to sand or straw greatly reduced the coliform infection rate. Paraformaldehyde treatment of bedding also seemed to be of some value in reducing coliform levels, which might be especially useful at critical times, such as shortly before and after parturition. He found that the udder became susceptible to E. coli infection 2 days before calving and remained so during lactation.

Anderson (1972; 1974) has applied a mouse model to the study of bovine mastitis. In his 1972 experiments, he found that teat damage, suckling, and the pathogenicity of the invading organism may all be important factors in the production of mastitis. In 1974, he attempted to determine whether more than one strain of an organism might establish in separate quarters of the same udder simultaneously. He discovered that a Staphylococcus aureus strain of high virulence in one gland enhanced the

virulence of a strain of low virulence in another gland. He believed that the presence of one virulent strain may have reduced the effectiveness of the systemic nonspecific defense mechanism, leaving it less able to cope with the low virulence strain.

Gilruth and MacDonald (1911) were among the first to point out that milk from early stages of infection is favorable for the growth of coliform bacteria, but that it rapidly becomes inhibitory to the organisms.

Carroll et al. (1963) reported that increased vascular permeability in the mammary gland (increased pH, chlorides, serum albumin, and immune globulin) was noted within 3 hours after intramammary inoculation of Klebsiella organisms. Normal permeability was quickly restored, but the immune globulin fraction remained elevated. Nonspecific circulating antibodies increased in amount following the udder inoculation, but specific antibodies to the organism or its endotoxin were not found in the blood or milk.

Schalm et al. (1964a,b,c) and Carroll et al. (1964) carried out a series of experiments to determine why coliform mastitis isn't more prevalent in view of the ubiquity and potential pathogenicity of the organisms. They described the pathogenesis of mastitis produced by injection of approximately 100 viable K. pneumoniae into a normal udder. The number of organisms reisolated fell rapidly when leukocytes first infiltrated the gland. If a pre-existing leukocytosis was present in the gland, the organisms did not survive. The authors were able to simulate coliform mastitis by infusion of K. pneumoniae endotoxin into the udder

and postulated that endotoxin released as the bacteria were phagocytized was the causative factor in acute coliform mastitis.

Blobel and Katsube (1964) and Katsube and Blobel (1964) reported that experimental leukocytosis caused by intramammary infusion of 0.14M sodium chloride made mammary glands resistant to infection by K. pneumoniae. They believed that polymorphonuclear leukocytes could be only a part of the udder defense mechanism, however, because to a great extent K. pneumoniae survived phagocytosis by these cells.

Jain et al. (1967a) and Collins et al. (1969) found that cell-free normal milk supernates from normal and immunized cows were nonbactericidal for K. pneumoniae in vitro, but that cell-free mastitic milk supernates were highly bactericidal. The activity of normal serum and mastitic milk seemed to involve heat-labile natural antibody and complement components. They suggested that the decrease in bacterial counts in milk during the first 2 hours and slow increase thereafter was due to absorption of this humoral inhibitory component by the bacteria, which allowed the survivors to grow. Baumgartner et al. (1965), however, felt that this apparent initial inhibition was actually a lag in growth during adaptation to new growth substrates, since it could be overcome by subculturing the organisms in milk prior to inoculation into the test system.

While furthering the work of Jain et al., Carroll and Jain (1969) demonstrated that a large percentage of normal milk samples were bactericidal. All mastitic milk samples were bactericidal, half of them markedly so. Colostrum had only slight activity, even with added complement. They suggested that this natural antibody system is probably a reflection of the constant close association of most mammalian species with Gram-

negative antigens. That milk from normal glands was sometimes bactericidal seemed to indicate that certain glands respond more readily than others to minor trauma with the transfer of proteins from blood to milk.

Jain et al. (1967b) demonstrated that egg albumin-induced leukopenia would permit normally innocuous bacteria to multiply suddenly and produce acute disease. Jain et al. (1968) showed that unrestricted multiplication did not always occur with leukopenia induced by equine anti-bovine leukocyte serum (EABLS). They felt that this was probably due to the humoral bactericidal activity of milk. The lack of leukocytosis into the milk was paralleled by the absence of clinical signs of acute mastitis. Jain et al. (1971) found that unrestricted multiplication in these neutropenic cows, and the endotoxin released thereby, could lead to an extreme inflammatory reaction and tissue necrosis. Thus there was some evidence to indicate that a system other than neutrophil leukocyte mediation was capable of producing increased vascular permeability.

Salajka (1968), in a comparison of four healthy herds and two mastitic herds, found natural antibodies active against a K. pneumoniae mastitis strain in diseased cows, healthy cows, and calves only 1 month old. He did not know whether the antibodies resulted from exposure to K. pneumoniae itself or to related antigens in other organisms or feed.

Carroll et al. (1969) worked with two strains of K. pneumoniae, one of which, 2414-1, had been lyophilized and stored. Strain 2414-2, from this original culture, was maintained on laboratory mediums and reisolated from cows seven times. Strain 2414-1 was still able to produce mastitis and was resistant to the bactericidal action of milk. Strain 2414-2 was no longer able to produce mastitis and had become susceptible to the

bactericidal system. This indicated that at least one feature of virulence in the mammary gland is the relative capacity of an organism to survive the bactericidal effects of milk. Carroll (1971) was able to demonstrate that Klebsiella spp. highly resistant to serum bactericidal action exist in the environment of the cow, along with relatively susceptible strains. Different serums were shown to be variable in their activity against susceptible organisms.

Muschel (1960) found hundredfold variations in the susceptibility of E. coli strains to serum, but his evidence did not indicate that serum resistance was a basis for differentiating virulent from nonvirulent strains. Bramley (1974), however, proposed that the serum-sensitive: serum-resistant strain ratio may vary between herds, and that this may be of practical significance in determining the frequency of occurrence of acute mastitis.

Carroll et al. (1973) determined that serum-resistant strains produced acute mastitis, while serum-sensitive strains elicited no response or a mild transient mastitis. In 1974, Carroll (1974) mentioned that strains of the same organism with different degrees of serum susceptibility could be developed by subjecting them to different periods of storage in broth at room temperature. Michael and Landy (1961) obtained strains from the same culture that differed greatly in susceptibility by selecting colonies of different density and refractivity from the agar surface.

Antibacterial Systems in Milk

Antibacterial factors in bovine milk include leukocytes and inhibitors derived from the blood, as well as inhibitory substances synthesized locally by the mammary gland. Immunoglobulins, complement, and transferrin are derived from serum, and the secretory immunoglobulins, lactoferrin, and lysozyme are produced in the mammary gland.

All of these inhibitory factors are present in colostrum at higher concentrations than in milk. However, bovine colostrum has been reported to be neither bactericidal nor bacteriostatic for various Klebsiella and E. coli serotypes (Carroll and Jain, 1969; Wilson, 1972; Reiter and Brock, 1975). Reiter and Brock (1975) believed that this may be due to a prozone effect, and that bactericidal activity becomes manifest as concentrations of the inhibitors decrease during the change from colostrum to milk. Bullen et al. (1972) found that lactoferrin was capable of inhibiting E. coli in colostrum, but only after the addition of bicarbonate. Bramley (1974) confirmed Bullen's work and determined that this inhibitory system, while present both before and after calving, was inactive in colostrum due to an increased citrate concentration. He stated, in addition, that the leukocyte cell count in colostrum is lower than in dry secretion, and that this may play a role in susceptibility to infection after calving. He also mentioned that phagocytic efficiency may be low in colostrum.

Approximately 80% of the immunoglobulin in colostrum is of the IgG class, but the amounts of IgG and IgM decrease as the secretion changes to milk, which leaves IgA the predominant immunoglobulin in milk (Porter and Noakes, 1969). Secretory IgA in colostrum and milk has been shown to be

bactericidal for E. coli in the presence of lysozyme and complement. IgA is thought to activate the alternate complement system. IgM, which is the most efficient agglutinating and bactericidal immunoglobulin, requires complement, but not lysozyme, to kill E. coli (Porter and Noakes, 1969; Porter and Allen, 1972; Hill and Porter, 1974). Reiter and Brock (1975) found, however, that E. coli antibodies or complement or both may not be present in some milk samples, since this seems to vary with the individual cow.

The protein lactoferrin has been found in human and bovine neutrophilic leukocytes, milk, and colostrum. It is not present in serum. It reversibly binds iron atoms, thereby denying them to iron-requiring bacteria (Masson et al., 1969). Bicarbonate ions are necessary for the system to be active. Complement does not seem to be involved in the reaction, although specific antibodies are thought to be required. The system is stable to heating at 56°C. Its bacteriostatic action is destroyed when additional iron or large amounts of citrate are added to an in vitro test system, and it is inactive when high concentrations of citrate are present in vivo (Bullen et al., 1972; Bramley, 1974; Reiter et al., 1975). Glynn (1972) demonstrated that Klebsiella strains mixed with iron compounds were more virulent for rats, guinea pigs, and mice than were Klebsiella strains injected alone. Transferrin is an iron-binding protein in serum and milk with a bacteriostatic action similar to that of lactoferrin (Glynn, 1972; Bullen et al., 1972; Reiter et al., 1975).

Klebsiella pneumoniae

Klebsiella nomenclature and characteristics

Confusion has existed for years concerning the nomenclature of the genus Klebsiella. The organisms now known as Klebsiella pneumoniae have been called Bacillus lactis aerogenes, Aerobacter aerogenes, Friedlander's bacillus, Bacterium friedlanderi, coli-aerogenes group, Klebsiella-Aerobacter group, Klebsiella aerogenes, Klebsiella edwardsii, and Klebsiella oxytocum. All of those organisms in the literature conforming to the cultural and biochemical characteristics of K. pneumoniae as given in the 8th edition of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) will be referred to as such in this report.

The genus Klebsiella Trevisan is described as consisting of capsulated Gram-negative rods, which are differentiated from the genus Enterobacter by being nonmotile and ornithine decarboxylase negative. All Klebsiella strains which are not K. ozaenae or K. rhinoscleromatis are considered to be K. pneumoniae. The type species, K. pneumoniae (Schroeter) Trevisan, produces acid and gas from glucose and acid from lactose. Acid production from dulcitol varies. Citrate is utilized, and urease and lysine decarboxylase are produced. The methyl red test is negative, and the Voges-Proskauer test is positive. Indole and gelatinase are usually negative. Hydrogen sulfide is not formed in triple sugar iron agar.

There are 11 somatic or O antigens in the Klebsiella genus, but serological typing is primarily based on the capsular or K antigens.

There are 80 known K antigens, which are determined by the Quellung reaction, and all can be found within the species K. pneumoniae (Ørskov, 1974).

Klebsiella pneumoniae in the environment

Klebsiella pneumoniae is widely found in nature, as well as being a part of the normal flora in the intestine of man and animals (Nieva, 1971; Ørskov, 1974). In a survey of K. pneumoniae from animals and their environment, Nieva (1971) found the organisms more prevalent in drinking water than any other source, in swine environment more than in those of any other species, and in soil from farms without livestock more than in soil from public parks. There was a higher incidence of isolation from swine, dairy cattle, and horses than from sheep and beef cattle. K. pneumoniae was widespread in surface soil, but not in soil from more than 1 meter below the surface. Nieva points out that, although the organism can live apart from an animal host, when it does infect or colonize an animal, it is shed in large numbers, thereby increasing the population in the environment.

Klebsiella spp. have been isolated from fresh water and drinking water (Henriksen, 1954), trees from forests and paper mills (Duncan and Razzell, 1972), and vegetables and seeds (Duncan and Razzell, 1972; Brown and Seidler, 1973). Thomas et al. (1960) reported the presence of Klebsiella spp. in surface soil of both heavily grazed pastures and enclosed forestry plantations. There were much higher numbers in the unpolluted soil in summer than in winter.

Feary et al. (1972) found antibiotic-resistant coliforms in fresh and salt water and theorized that they originated in the drain-off wastes from

livestock feedlots. There was a high incidence of streptomycin and tetracycline resistance in coliforms isolated near the feedlots, probably as a direct result of the use of these antibiotics in animal feed and water. Sturtevant and Feary (1969) also believed this to be a possible cause of the antibiotic-resistance in Klebsiella spp. and E. coli they isolated from both raw and treated sewage.

Matsen et al. (1974) compared strains of K. pneumoniae from fresh and salt water with hospital-associated human strains. The biochemical reactions, serotype distribution, and mouse pathogenicity tests revealed no difference between the two groups. The environmental isolates, however, were sensitive to most antibiotics, while the hospital strains showed patterns of multiple resistance. They concluded that Klebsiella spp. are widely distributed in nature, and that human colonization likely occurs from a variety of sources.

Nieva (1971) also reported that strains isolated from animals were more resistant to antibiotics than those from sources unrelated to animals. He believed that this may have reflected antibiotic usage in the treatment of animals.

Klebsiella pneumoniae in hospitals

Klebsiella pneumoniae is assuming a position of increasing importance in nosocomial infections, especially as the cause of urinary infection and septicemia. The majority of K. pneumoniae infections are considered to be hospital acquired (Steinhauer et al., 1966; W. J. Martin et al., 1971). Klebsiella spp. can be the most frequent cause of septicemia in hospitals, and the mortality is usually around 50% of those affected (Steinhauer et al., 1966; Davis and Matsen, 1974). It is clear that more than one

serotype can cause disease, but prevalent serotypes are often isolated from individual outbreaks of serious infection (Steinhauer et al., 1966; Gardner and D. Smith, 1969; Selden et al., 1971; Davis and Matsen, 1974).

In several outbreaks of K. pneumoniae disease, the source of the organisms could be traced to the patient's environment. Klebsiella spp. have a selective ability to survive and perhaps multiply in aerosol solutions (Mertz et al., 1967; Walter, 1974), intravenous dextrose solutions (Maki and W. T. Martin, 1975), hand lotions (Morse et al., 1967), and on floors, tables, linens, and in the air (Turner and Salmonsen, 1973; Walter, 1974). Salzman et al. (1967) demonstrated that over 20% of hospital personnel examined had antibiotic-resistant coliforms on their hands, frequently in large numbers. Montgomerie et al. (1970), Shooter et al. (1971), and Clinical Laboratory Forum (1973) reported the isolation of Klebsiella spp. from food in large enough numbers to change the fecal flora if ingested. The organisms were most commonly isolated from salads and vegetables, though milk products seemed to promote their multiplication very well.

Pollack et al. (1972) reported that the number of patients with Klebsiella spp. in their throats or on their hands increased markedly during antibiotic therapy. Others have noted the increase in Klebsiella infections in patients with prior antibiotic therapy (McCabe and Jackson, 1962; Gardner and D. Smith, 1969). Several authors have found that patients placed on antibiotic therapy rapidly became intestinal carriers of K. pneumoniae (Salzman et al., 1967; Montgomerie et al., 1970; R. Smith et al., 1973). Selden et al. (1971) felt that antibiotic-induced intestinal colonization may lead to infection with the organisms and may be a

significant reservoir of K. pneumoniae in the hospital. They and Montgomerie et al. (1970) were able to correlate the fecal strain or serotype of K. pneumoniae with that isolated from the subsequent infection.

Price and Sleigh (1970) recorded a severe outbreak of Klebsiella pneumonia, urinary infection, and meningitis in a neurosurgical ward. The increase in numbers of K. pneumoniae isolated from infections, personnel, and environment corresponded to an increase in consumption of antibiotics. Working under the hypothesis that Klebsiella is an opportunist ready to colonize patients whose bacterial flora was suppressed, administration of all antibiotics was forbidden on this ward. The Klebsiella infection rate fell to zero, and the number of infections caused by other bacteria also decreased.

MATERIALS AND METHODS

Pathogenicity Determinations in Swine

Experimental animals

All experimental animals were second-litter Landrace or cross-bred sows obtained at 108 to 112 days of gestation.[1] They were housed in isolation units in farrowing crates. Wood shavings were used for bedding. Prior to farrowing, they were fed 4 to 6 pounds per day of 16% protein gestation-lactation ration without added antibiotics. After farrowing, the same ration was fed in increasing amounts until the sows were on full feed (approximately 12 pounds per day).

Experimental procedures

Experimental procedures with all sows were begun 24 to 48 hours after farrowing.

Milk samples were obtained by injecting 1 to 2 cc of oxytocin[2] into the marginal ear vein. Teats were cleaned with cotton balls soaked in 70% (vol/vol) ethanol. After discarding the first few streams of milk, approximately 1 ml was collected from each gland in a sterile plastic tube.[3] Preinoculation samples were taken at least 1 hour before inoculations were made.

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[1]Obtained from Swine Nutrition Farm, Iowa State University, Ames, Ia.

[2]Armour-Baldwin Laboratories, Omaha, Neb.

[3]No. 2057 tube, Falcon Plastics, Los Angeles, Calif.

Each milk sample (0.05 ml) was streaked onto three media: tryptose agar base[1] plus 5% (vol/vol) defibrinated horse blood (BA); Tergitol-7 agar[1] plus 0.04% (wt/vol) triphenyl tetrazolium chloride (T7); and MacConkey agar[1] (MAC). When colony-forming units (CFU) were determined, tenfold dilutions of the milk were made in sterile 0.85% (wt/vol) NaCl, and three 0.05 ml aliquots of the 10^{-1} and 10^{-3} dilutions were inoculated on MAC agar. All plates were incubated overnight at 37°C in a high humidity, water-jacketed incubator.[2]

Identification of Klebsiella was based on colony morphology, Gram's stain reaction, microscopic morphology, cytochrome oxidase[3] reaction, and Enterotube[4] reactions. Occasionally, coliforms other than Klebsiella were isolated from a milk sample. In such instances, it was assumed that the gland had an existing coliform infection, and it was excluded from the test. The presence of other bacteria in the milk, such as beta-hemolytic streptococci and micrococci, was ignored.

The pH of each milk sample was determined with a Zeromatic II pH meter.[5] Somatic cells in milk were counted in films stained with the Levowitz-Weber modification of the Newman-Lampert stain (Schalm et al., 1971, p. 99).

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[1]Difco Laboratories, Detroit, Mich.

[2]National Appliance Co., Portland, Ore.

[3]PathoTec, General Diagnostics, Morris Plains, N.J.

[4]Roche Diagnostics, Nutley, N.J.

[5]Beckman Instruments, Inc., Fullerton, Calif.

Experiment 1

The first experiment was carried out in sow 1 to determine which route of inoculation would promote establishment of organisms in the mammary glands. A log phase culture of strain M1 growing in tryptose phosphate broth[1] (TPB) was used for each method.

Method 1: 0.5 cc of culture was injected through the skin into the parenchyma of the anterior half of each of two glands.

Method 2: 0.5 cc of culture was inoculated into each of two anterior teat canals (IMM) with a 22 gauge, 1-inch cannula. Cannulas were prepared by blunting the end of disposable 22 gauge needles. They were then rinsed with alcohol followed by sterile 0.85% NaCl.

Method 3: a cotton-tipped applicator was dipped in culture and rubbed over each of two teat orifices.

Method 4: each of two teats was dipped into the culture.

At 4 hours postinoculation (PI) all the teats were swabbed with sterile cotton-tipped applicators, oxytocin was given, and milk samples were collected. Swabs and milk samples were streaked on BA, T7, and MAC plates.

Experiment 2

Experiment 2 was carried out to determine whether organisms grown in the yolk sac of embryonated hens' eggs would establish better in the mammary gland than those grown in TPB. Yolk sacs were inoculated with strain M1, and the fluids were harvested after 24 hours incubation at 37°C. The material was streaked on BA and MAC agar to check for purity,

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[1]Difco Laboratories, Detroit, Mich.

and aliquots were stored at -70°C. Strain M1 was grown 24 hours in TPB and stored at -70°C. The TPB-grown strain M1 had 4.4 x 10^8 CFU/ml, and the yolk sac fluid 3.3 x 10^8 CFU/ml. The TPB culture was diluted so that it contained the same number of viable organisms as the egg yolk culture, and 0.5 cc of each preparation was inoculated IMM (with a 22 gauge, 1-inch cannula) into two glands of sow No. 2. Milk samples were collected preinoculation (PRE) and 4 and 24 hours PI.

Experiments 3, 4, and 5

Three experiments were conducted to determine the number of organisms required to establish infection, but not cause a severe inflammatory response. It was assumed that a large dose of even a low virulence strain would elicit inflammation due to the high content of endotoxin and other cell constituents.

In Experiment 3 (sow 3), 0.25 cc of a 4.4 x 10^8 CFU/ml culture of strain M1 grown in TPB was injected into the anterior and posterior teat canals of each of two glands. This was repeated with cultures containing 4.4 x 10^6 CFU/ml and 4.4 x 10^4 CFU/ml, using two glands for each dilution. Milk samples were collected PRE and 2, 8, 24, and 48 hours PI. In Experiments 4 and 5 (sows 4 and 5), the same procedure was followed using cultures diluted to contain 4.4 x 10^6 CFU/ml, 4.4 x 10^4 CFU/ml, and 4.4 x 10^2 CFU/ml.

Experiments 6, 7, 8, 9, and 10

Experiments 6, 7, 8, and 10 were conducted in four sows to compare the establishment of K. pneumoniae strain M1, isolated from a case of mastitis, with K. pneumoniae strain E32, isolated from the environment (wood shavings prior to use as sows' bedding). Strain M1 and strain N25,

isolated from the milk of a normal sow, were compared in Experiment 9 (sow 9).

Teats were numbered M1 through M7, anterior to posterior, and R or L for the sow's right or left side.

Strain M1 (0.25 cc of a 4.4 x 10^4 CFU/ml culture) was inoculated into each of the two teat orifices of glands M1R, M3R, and M5R. Strain E32 (0.25 cc of a 3.9 x 10^4 CFU/ml culture) or strain N25 (0.25 cc of a 4.4 x 10^4 CFU/ml culture) was inoculated into each orifice of glands M1L, M3L, and M5L. M2R and M2L were used as controls and were not inoculated. PRE, 2, 8, 24, and 48 hour PI milk samples were collected in Experiment 6, and PRE, 2, 8, 24, 48, 72, 96, and 120 hour PI samples were collected in Experiments 7, 8, 9, and 10.

In vitro Comparisons

Experimental K. pneumoniae strains

Forty-four strains of K. pneumoniae were compared (Table 1). Seventeen strains had been isolated from mastitic or normal swine during the 8 year period from 1967 through 1974. Seven strains were isolated from mastitic swine in a herd (Schoenfelder Farm) that had an outbreak of coliform mastitis in June and July of 1973. Two more strains were isolated from wood shavings prior to use as bedding in this herd. Four strains were reisolated from swine inoculated as part of this project (Experiments 2, 7, 8, and 9). Three strains from mastitic swine were

Table 1. Origin of Klebsiella pneumoniae strains

Strain No.	Original source	Animal and/or specimen No.	Clinical status	Date of isolation
M1	Mammary gland	Sow 3013 M3	Mastitis	5-10-68
M2	Milk	Sow A2 M1L	Reisolated 24 hours PI from M1 inoculated gland	3-15-74
M3	Milk	Sow A7 M5R	Reisolated 120 hours PI from M1 inoculated gland	7-11-74
M4	Mammary gland	Sow 1040 M1-A	Mastitis	11-16-67
M5	Mammary gland	Sow 1040 M1-B	Mastitis	11-16-67
M6	Superficial inguinal lymph node	Sow 1040 LN-A	Mastitis	11-16-67
M7	Superficial inguinal lymph node	Sow 1040 LN-B	Mastitis	11-16-67
M8	Mammary gland	Sow 2781 M11	Mastitis	1-23-68
M9	Superficial inguinal lymph node	Sow 2781	Mastitis	1-23-68
M10	Mammary gland	Schoenfelder sow 10 M6L	Mastitis	7-6-73

Table 1. (Continued)

Strain No.	Original source	Animal and/or specimen No.	Clinical status	Date of isolation
M11	Superficial inguinal lymph node	Schoenfelder sow 10	Mastitis	7-6-73
M12	Milk	Schoenfelder sow 15-1M5L	Mastitis	7-6-73
M13	Milk	Schoenfelder sow 16-1M3L	Mastitis	7-6-73
M14	Milk	Schoenfelder sow 16-1M2R	Mastitis	7-6-73
M15	Vaginal secretions	Schoenfelder sow 16B	Mastitis	7-6-73
M16	Milk	Schoenfelder sow 17-1M1L	Mastitis	7-6-73
M17	Uterus	Sow 72-2	Mastitis	9-20-72
M18	Uterus	Sow 74-18	Mastitis	1-24-74
M19	Mammary gland	Sow 74-19 M4R	Mastitis	2-4-74
M20	Superficial inguinal lymph node	Sow 74-19	Mastitis	2-4-74
M21	Mammary gland	Sow 4511 M7	Mastitis	7-21-70

Table 1. (Continued)

Strain No.	Original source	Animal and/or specimen No.	Clinical status	Date of isolation
M22[a]	Mammary gland	Sow 11 M12	Mastitis	11-17-72
M23[a]	Mammary gland	Sow 11 M15	Mastitis	11-17-72
M24[a]	Mammary gland	Sow 11 M26	Mastitis	11-17-72
N25	Milk	Sow 3061 M1	Normal	10-7-68
N26	Milk	Sow 3061 M5	Normal	10-7-68
N27	Nasal secretions	Sow 3061	Normal	10-7-68
N28	Milk	Sow A9 M5L	Reisolated 24 hours PI from N25 inoculated gland	7-8-74
N29	Nasal secretions	Swine Nutrition Pig 9	Normal	11-26-74
N30	Milk	Sow 2G M2R	Normal	1-4-74
N31	Vaginal secretions	Sow 3G	Normal	1-8-74

[a]Provided by Dr. J. S. McDonald.

Table 1. (Continued)

Strain No.	Original source	Animal and/or specimen No.	Clinical status	Date of isolation
E32	Unused wood shavings	Schoenfelder Farm (smooth colony)	N.A.[b]	7-6-73
E33	Unused wood shavings	Schoenfelder Farm (mucoid colony)	N.A.	7-6-73
E34	Milk	Sow A8 M5L	Reisolated 96 hours PI from E32 inoculated gland	7-11-74
E35	Unused wood shavings	Swine Nutrition Farm (indole +)	N.A.	11-26-74
E36	Unused wood shavings	Swine Nutrition Farm (indole -)	N.A.	11-26-74
E37	Water	Swine Nutrition water bowl 4	N.A.	11-26-74
E38	Water	Swine Nutrition water bowl 7	N.A.	11-26-74
E39	Feed	Swine Nutrition sow 3	N.A.	11-26-74

[b]N.A. = not applicable.

Table 1. (Continued)

Strain No.	Original source	Animal and/or specimen No.	Clinical status	Date of isolation
E40	Feed	Swine Nutrition sow 7	N.A.	11-26-74
B41[c]	Mouth	Bovine K-6	Normal	1961
B42[c]	Milk	Bovine K-1	Mastitis	1961
B43[c]	Milk	Bovine 2414-1	Mastitis	1950
B44[c]	Milk	Bovine 2414-2	Mastitis	1950

[c]Provided by Dr. E. J. Carroll.

kindly provided by Dr. J. S. McDonald[1], and four from bovine sources were provided by Dr. E. J. Carroll.[2]

Seven of the strains (Swine Nutrition Farm) were isolated from the farm that supplied experimental animals for this project. Samples were collected from swine (nasal and vaginal secretions, rectal contents, udder skin, and teat orifices) with sterile cotton-tipped swabs and streaked on BA, T7, and Simmons citrate agar[3] (SC) plates. Feed and bedding were added in 2 gram amounts to 25 ml of lauryl sulfate broth[4], and the broth was incubated at 37°C for 18 hours. These cultures were then streaked on BA, T7, and SC. Colonies suspected of being Klebsiella were identified as described previously.

Three E. coli strains were often included as test controls. Strains S16 and Lilly were obtained through the courtesy of Dr. E. J. Carroll. When streaked on BA, strain Lilly dissociated into smooth and rough forms. Colonies of each type were selected and referred to as strains Lilly S and Lilly R.

Biochemical characterization

All K. pneumoniae strains were characterized by their reactions in Enterotubes. Further comparisons were made in phenol red broth base[3] plus 0.5% (wt/vol) sucrose or dulcitol, and in malonate broth.[3] Reactions

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[1]National Animal Disease Center, Ames, Ia.

[2]University of California, Davis, Calif.

[3]Difco Laboratories, Detroit, Mich.

[4]Baltimore Biological Laboratories, Cockeysville, Md.

in methyl red-Voges Proskauer broth[1] and sulfide-indole-motility agar[1] were determined using the procedures indicated by the supplier.

All isolates were stored at room temperature on tryptose agar[1] slants with rubber stoppers.

Capsule size determination

All cultures were streaked on Worfel-Ferguson agar[1] and incubated at 37°C for 18 hours. The organisms were then suspended in 0.5% (vol/vol) formaldehyde and diluted so that the density equaled that of a standard prepared by adding 0.3 ml of 1% (wt/vol) $BaCl_2$ to 9.7 ml of 1% (vol/vol) H_2SO_4 (0.36 N). A small drop of the cell suspension was placed on a microscope slide next to a drop of Capsule Ink.[1] A coverslip was placed over the two drops so that they mixed, and the preparation was allowed to stand for 1 hour before examination at 970X under an oil-immersion objective. Capsule sizes were estimated by comparing their width to the diameter of the cell. Noncapsulated cultures were recorded as zero. A capsule appearing to be the same size as the cell width was recorded as 1. The largest capsules appeared to be twice the size of the cell diameter and were recorded as 2.

Antibiotic sensitivity

The standardized single disc method of Bauer et al. (1966) was used to compare the sensitivity of K. pneumoniae strains to seven antibiotics. The cultures were grown in TPB until equal in density to a standard of 0.5 ml of 1% $BaCl_2$ in 99.5 ml of 1% H_2SO_4 (0.36 N). The standardized suspensions were streaked on freshly prepared Mueller-Hinton agar.[1] Discs

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[1]Difco Laboratories, Detroit, Mich.

were dispensed, and the plates were incubated at 37°C for 18 hours. The zone diameters were read with a Fisher-Lilly Antibiotic Zone reader.[1] Each culture was categorized as resistant, intermediate, or sensitive to each drug, according to criteria established by Bauer et al. (1966) (Table 2).

Hemagglutination

The procedures of Old (1972) were used to compare hemagglutinating properties of K. pneumoniae strains. The cultures were incubated in TPB aerobically without shaking for 48 hours at 37°C. Three more subcultures were made in this manner, each time transferring a loopful of broth pellicle into fresh TPB. The CFU/ml of the last subculture was determined, and the cultures were killed by 0.1% (vol/vol) formaldehyde and stored at 4°C.

Guinea pig blood was obtained by cardiac puncture, and 4 ml were added to 6 ml of Alsever's solution. Erythrocytes were centrifuged, washed once, and diluted to 1% (vol/vol) with sterile 0.85% NaCl.

The bacterial suspension to be tested (0.5 ml) and the red cell suspension (0.5 ml) were mixed in a 6.5 x 1 cm tube and left overnight at 4°C. Agglutination was indicated by a diffuse carpet, rather than a discrete button, of red cells on the bottom of the tube.

The Minimum Hemagglutinating Dose (MHD) of a culture was the smallest number of bacteria per ml that resulted in visible hemagglutination. The final Hemagglutinating Power (HP) was determined by dividing 10^{11} by the MHD. E. coli strain S16 was included as a positive control in each test.

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[1] Fisher Scientific Co., Chicago, Ill.

Table 2. Zone sizes and their interpretation for seven antibiotics[a]

Antibiotics[b]	Disk potency	Inhibition zone diameter[c]		
		Resistant	Intermediate	Sensitive
Ampicillin	10 µg	11 or less	12-13	14 or more
Cephalothin	30 µg	14 or less	15-17	18 or more
Chloramphenicol	30 µg	12 or less	13-17	18 or more
Kanamycin	30 µg	13 or less	14-17	18 or more
Penicillin-G	10 units	20 or less	21-28	29 or more
Streptomycin	10 µg	11 or less	12-14	15 or more
Tetracycline	30 µg	14 or less	15-18	19 or more

[a]Bauer et al., 1966.

[b]Baltimore Biological Laboratories, Cockeysville, Md.

[c]Zone diameter in mm.

Serum and milk sensitivity tests

Blood was drawn by anterior vena cava puncture from normal sows and allowed to clot. The serum was harvested and sterilized through either a 0.45 µm cellulose acetate[1] or a Selas 02 porcelain[2] filter. It was stored at -20°C.

Cell-free mastitic milk was obtained using a modification of the procedure of Jain et al. (1967a). Mastitis was induced in a normally lactating sow by injecting approximately 6 ml of sterilized distilled

[1]Gelman Instrument Co., Ann Arbor, Mich.

[2]Selas Corporation of America, Spring House, Pa.

water into each teat. An injection of oxytocin was given 12 hours later, and milk was collected from all glands and chilled in an ice bath. It was then filtered through gauze and centrifuged in a refrigerated centrifuge[1] at 1000 x g for 30 minutes. The whey was removed from between the upper fat and lower cell-rich layers, sterilized by filtration[2], and stored at -70°C.

Sensitivity test procedures were modifications of those of Carroll and Jain (1969). The milk or serum was mixed with a 10^{-3} dilution of an overnight growth of the organism to be tested, in a ratio of 1 part bacterial suspension to 9 parts milk or serum. The mixtures were incubated in a 37°C water bath, and the number of CFU/ml was determined after 0, 2, and 5 hours incubation. A series of tenfold dilutions was made in sterile 0.85% NaCl kept in an ice bath. Six 0.1 ml aliquots of the appropriate dilutions were placed in the cups of plastic trays[3], which had been sterilized by ultraviolet light for at least 30 minutes. Two ml of molten (50°C) tryptose agar[4] containing 0.005% triphenyl tetrazolium chloride was then added to each cup. The plates were covered with plastic wrap and incubated overnight at 37°C, after which colonies were counted. Different dilutions were required, depending on the stage of incubation of the test system. At 0 hours, aliquots of 10^{-2}, 10^{-3}, and 10^{-4} dilutions

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[1]Model RC-2B, Ivan Sorvall Inc., Newtown, Conn.

[2]Selas Corporation of America, Spring House, Pa.

[3]96 WS Disposo Trays, Linbro Chemical Co., Inc., New Haven, Conn.

[4]Difco Laboratories, Detroit, Mich.

were used. At 2 and 5 hours incubation, a greater range was required (undiluted, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} dilutions), because some strains increased while others decreased in numbers.

Statistical Analyses

Means were calculated according to strain source and compared by analysis of variance. The F test was used to determine the significance of source variations.

RESULTS

Pathogenicity Determinations in Swine

In Experiment 1, no Klebsiella were recovered from samples collected with swabs. The milk sample from one injected gland (method 1) contained approximately 200 CFU/ml of K. pneumoniae. No Klebsiella were isolated from the other injected gland. Each of the glands inoculated IMM (method 2) yielded 70 to 150 CFU/ml of K. pneumoniae. Therefore, the IMM route, using a 22 gauge, 1-inch cannula, was used for all further experiments.

In Experiment 2, comparison was made of the ability of strain M1 grown in yolk sacs and in TPB to establish in the sow mammary gland. Milk collected 4 hours PI from glands inoculated with both cultures yielded approximately 7,000 CFU/ml of K. pneumoniae. At 24 hours PI, milk from glands inoculated with the egg yolk culture contained approximately 400 CFU/ml, while that from TPB culture inoculated glands contained approximately 200 CFU/ml of K. pneumoniae. Since there was little apparent difference between the two groups in number of organisms reisolated, TPB cultures were used in all further experiments.

Results of Experiments 3, 4, and 5 indicated that strain M1 established when inocula containing 4.4×10^4 CFU/ml were used, but that little inflammatory response resulted. Figure 1 illustrates CFU counts obtained from milk samples in Experiment 4. Inocula containing 4.4×10^4 CFU/ml were used in all further experiments.

In Experiments 6, 7, 8, 9, and 10, the pathogenicity of a K. pneumoniae strain isolated from mastitic milk (M1) was compared with that of two other K. pneumoniae strains; one isolated from wood shavings (E32) and one

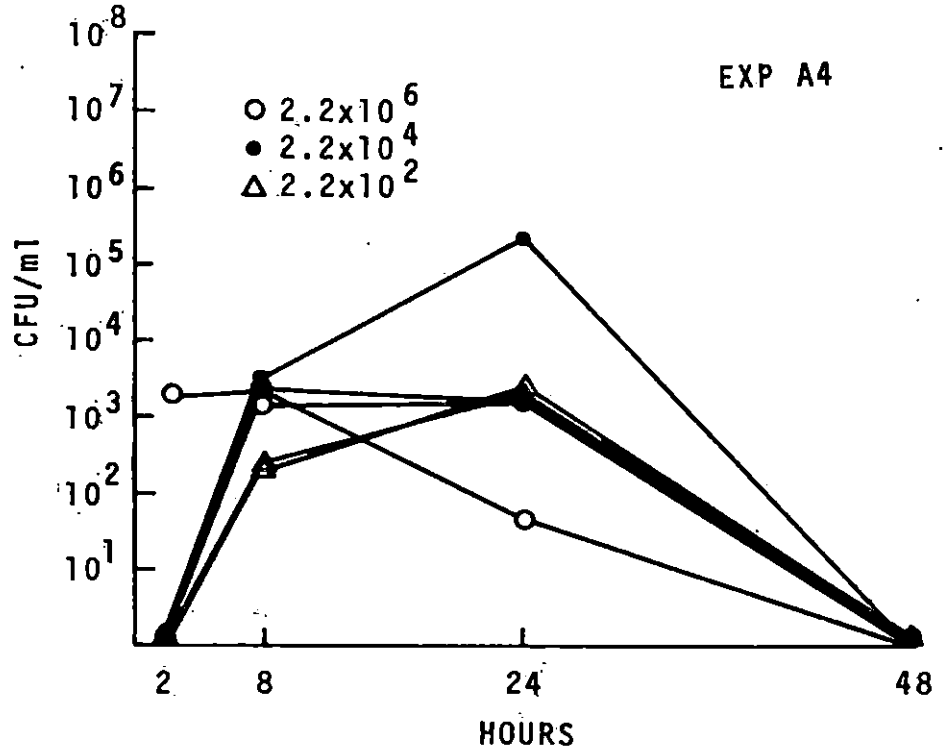

Figure 1. K. pneumoniae CFU count from milk of sow 4. M1R and M4L were inoculated with 2.2 x 10^2 CFU/ml of strain M1; M3R and M3L were inoculated with 2.2 x 10^4 CFU/ml; M5R and M2L were inoculated with 2.2 x 10^6 CFU/ml.

isolated from normal milk (N25). Mean pH values for milk collected from glands inoculated with M1 and E32 are presented in Table 3. There was a trend of increasing pH with successive sample intervals, but there were no significant differences in mean pH between the two groups.

Mean somatic cell counts in milk from glands inoculated with strains M1 and E32 are presented in Table 4. Cell counts were higher in milk of glands inoculated with M1 than in milk of glands inoculated with E32 at 24, 48, 72, 96, and 120 hours PI. The only difference that was significant was at 72 hours PI.

Figures 2 and 3 are representative of the K. pneumoniae CFU counts obtained from sows 8 and 9. The difference in numbers of organisms isolated from glands inoculated with M1 and E32 or N25 was most evident from 2 to 48 hours PI. At 72 hours, Klebsiella were isolated from previously negative inoculated glands, as well as some uninoculated control glands.

Means for K. pneumoniae CFU counts from glands inoculated with M1 and E32 are presented in Table 5. The number of organisms reisolated from glands inoculated with strain M1 was significantly higher than the number from E32 glands both at 8 and 24 hours PI. After 48 hours, the means fluctuated, and no trends could be observed. In fact, at 96 hours PI, the E32 mean was significantly higher than the M1 mean.

In vitro Comparisons

Biochemical characterization

Heterogeneity was detected among the 44 strains of K. pneumoniae by comparison of their biochemical reactions. The biochemical tests were divided into three groups, and various combinations of reactions in each

Table 3. Comparisons of pH of milk from glands inoculated with K. pneumoniae strains M1 and E32

		PRE	2 hours PI	8 hours PI	24 hours PI	48 hours PI	72 hours PI	96 hours PI	120 hours PI
Strain M1	Mean pH	6.56	6.55	6.65	6.95	6.75	6.92	7.03	7.13
	n[a]	11	11	11	11	11	8	8	8
Strain E32	Mean pH	6.55	6.55	6.62	6.90	6.76	6.87	6.97	6.93
	n	11	11	11	11	10	8	8	8
	SD[b]	0.06	0.05	0.04	0.10	0.07	0.09	0.17	0.21
	d.f.[c]	10	10	10	10	9	7	7	7

[a] n = number tested.

[b] SD = standard deviation.

[c] d.f. = degrees of freedom.

Table 4. Comparison of somatic cell counts in milk from glands inoculated with K. pneumoniae strains M1 and E32

		PRE	2 hours PI	8 hours PI
Strain M1	Mean somatic cell count[a]	4,880,000	13,360,000	6,597,000
	n[b]	14	14	13
Strain E32	Mean somatic cell count	7,062,000	11,290,000	7,050,000
	n	11	11	11
	SD[c]	0.33	0.16	0.23
	d.f.[d]	10	10	9

[a]Number of cells per ml of milk.

[b]n = number tested.

[c]SD = standard deviation.

[d]d.f. = degrees of freedom.

**The difference between the two means is significant at $P<0.01$.

24 hours PI	48 hours PI	72 hours PI	96 hours PI	120 hours PI
30,070,000	21,170,000	19,650,000	15,080,000	9,445,000
14	13	10	10	11
12,020,000	9,696,000	5,218,000	8,426,000	4,173,000
11	10	8	8	8
0.69	0.59	0.27	0.60	0.45
10	8	6 **	6	7

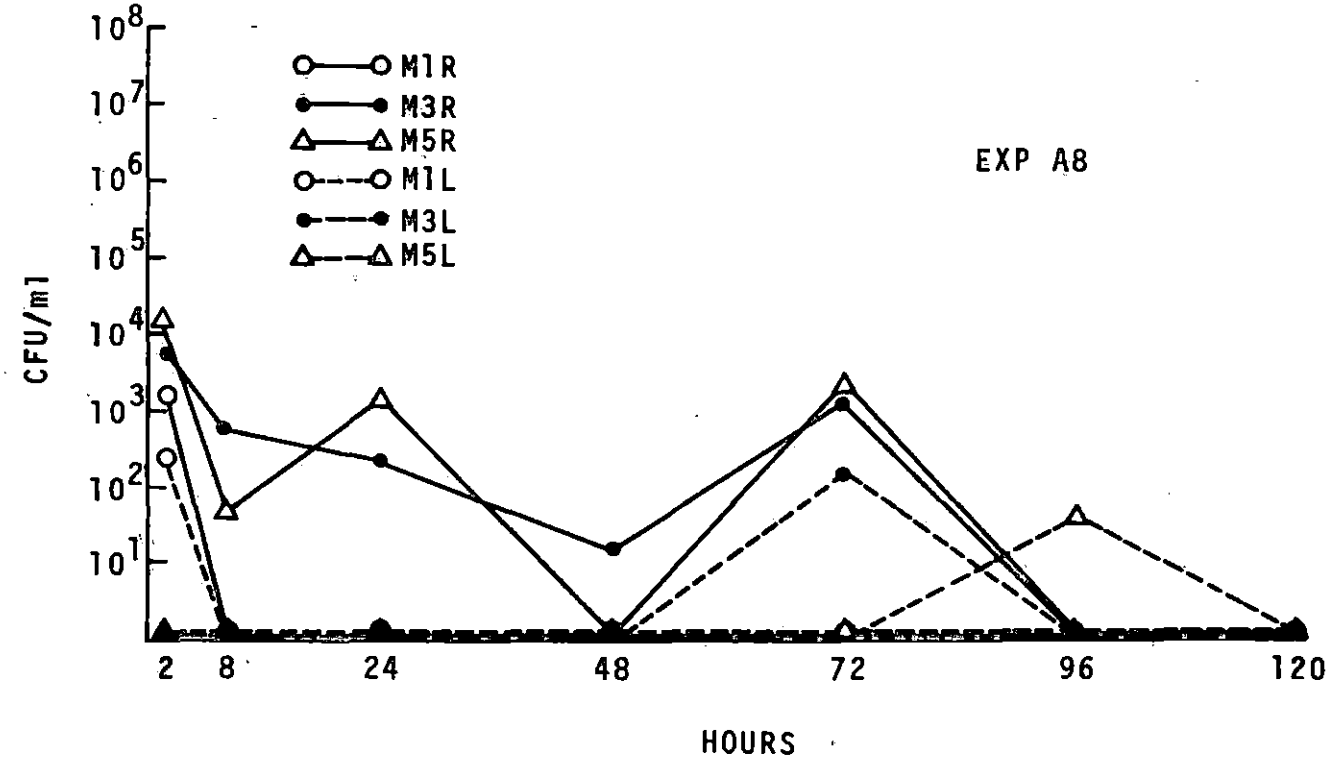

Figure 2. K. pneumoniae CFU count from milk of sow 8. M1R, M3R, and M5R were inoculated with strain M1, and M1L, M3L, and M5L were inoculated with strain E32.

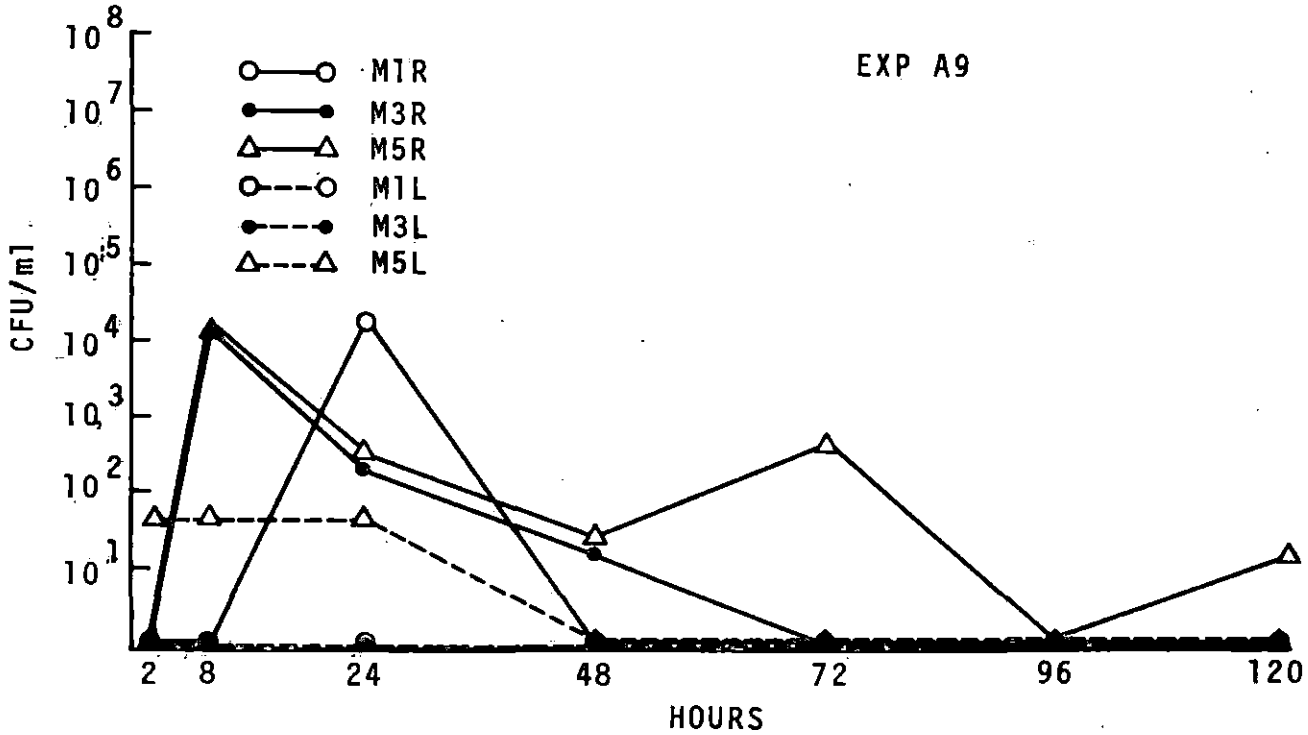

Figure 3. K. pneumoniae CFU count from milk of sow 9. M1R, M3R, and M5R were inoculated with strain M1, and M1L, M3L, and M5L were inoculated with strain N25.

Table 5. Comparison of K. pneumoniae CFU counts in milk from glands inoculated with strains M1 and E32

		PRE	2 hours PI	8 hours PI	24 hours PI	48 hours PI	72 hours PI	96 hours PI	120 hours PI
Strain M1	Mean CFU/ml	0	100.90	319.30	94.06	13.49	13.49	1.40	6.57
	n[a]	14	14	14	14	14	11	11	11
Strain E32	Mean CFU/ml	0	21.89	1.54	5.15	13.62	1.94	10.76	2.50
	n	11	11	11	11	11	8	8	8
	SD[b]	0	1.32	0.92	1.42	1.11	1.05	0.53	1.13
	d.f.[c]	10	10	10 **	10 *	10	7	7 **	7

[a] n = number tested.

[b] SD = standard deviation.

[c] d.f. = degrees of freedom.

** The difference between the two means is significant at $P<0.01$.

* The difference between the two means is significant at $P<0.05$.

group were numbered (Table 6). In this manner, similar to the classification of Rennie and Duncan (1974), a numerical biotype of three digits was assigned to each strain, and the 44 strains were placed in nine different biotypes. The majority of strains (35 of the 44) were either type 111 or 121, varying only in their ability to ferment dulcitol.

Capsule size determination

The mean capsule size was 1.56 for K. pneumoniae strains isolated from mastitic swine, 1.21 for strains from normal swine, and 1.50 for strains from the swine environment (SD 0.39, d.f. 37). There was no significant difference in mean capsule size among strains from the three sources.

Antibiotic sensitivity

The size of zones of inhibition obtained with seven antibiotics and K. pneumoniae strains from the three sources were compared (Table 7). Strains isolated from mastitic swine had significantly smaller zones of inhibition with ampicillin and penicillin than did strains from normal swine and swine environment. The mastitic swine strains also tended to have smaller zones with streptomycin, but this was not statistically significant. Strains from mastitic and normal swine had significantly smaller zones with cephalothin than did strains from the swine environment.

In a second comparison, zones of inhibition were used to classify K. pneumoniae strains as resistant, intermediate, or sensitive to the seven antibiotics (Table 8). All strains were sensitive to chloramphenicol and resistant to penicillin. A significantly greater proportion of

Table 6. Biotype classification of 44 K. pneumoniae strains

No. of strains	Group A					Group B	
	Indole	Methyl red	Voges Proskauer	Citrate	Code No.	Glucose acid and gas	Lactose
19	-	-	+	+	1	+	+
1	-	-	+	+	1	+	+
16	-	-	+	+	1	+	+
1	-	-	+	+	1	+	+
1	-	-	+	+	1	-[b]	+
2	-	+	-	+	2	+	+
1	+	-	+	+	3	+	+
1	+	-	+	+	3	+	+
2	+	+	-	+	4	+	+

[a]Biotype number derived from code numbers assigned to each set of reactions in Group A, B, and C.

[b]Acid, but no gas, produced from glucose.

			Group C					
Su-crose	Dul-citol	Code No.	Malon-ate	Ly-sine	Orni-thine	Urease	Code No.	Bio-type[a]
+	+	1	+	+	-	+	1	111
+	+	1	+	-	-	+	2	112
+	-	2	+	+	-	+	1	121
+	-	2	+	-	-	+	2	122
+	-	3	+	-	-	+	2	132
+	-	2	+	+	-	+	1	221
+	+	1	+	+	-	+	1	311
+	-	2	+	+	-	+	1	321
+	-	2	+	+	-	+	1	421

Table 7. Comparison of zone sizes obtained with seven antibiotics tested against K. pneumoniae strains from three sources

Antibiotics	Mean zone size of strains from three sources: Mastitic swine (n^a=24)	Normal swine (n=7)	Swine environment (n=9)	SD[b]	d.f.[c]
Ampicillin**	10.3[d]	14.0	13.8	2.35	37
Cephalothin*	19.5	19.5	22.2	2.35	37
Chloramphenicol	25.4	24.8	25.2	2.17	37
Kanamycin	19.6	22.1	21.2	4.82	37
Penicillin-G*	6.8	9.2	8.7	2.53	37
Streptomycin	10.9	14.6	14.8	5.57	37
Tetracycline	12.8	13.8	14.6	7.11	37

[a] n = number tested.

[b] SD = standard deviation.

[c] d.f. = degrees of freedom.

[d] Zone size measured in mm.

** Significant at P<0.01.

* Significant at P<0.05.

Table 8. Comparison of the proportion of K. pneumoniae strains from three sources that were resistant, intermediate, or sensitive[a] to five antibiotics

Antibiotics	Source of strains	No. of strains		
		Resistant	Intermediate	Sensitive
Ampicillin**	Mastitic swine	17	3	4
	Normal swine	0	2	5
	Swine environment	1	1	7
Cephalothin	Mastitic swine	1	3	20
	Normal swine	0	0	7
	Swine environment	0	0	9
Kanamycin	Mastitic swine	4	0	20
	Normal swine	0	0	7
	Swine environment	0	0	9
Streptomycin*	Mastitic swine	18	0	6
	Normal swine	2	0	5
	Swine environment	4	0	5
Tetracycline	Mastitic swine	14	1	9
	Normal swine	3	1	3
	Swine environment	4	0	5

[a]Classified according to criteria of Bauer et al. (1966).

**Significant at $P<0.01$.

*Significant at $P<0.05$.

strains from mastitic swine were resistant to ampicillin and streptomycin than were strains from the environment or normal swine.

Hemagglutination

The HP of E. coli strain S16 used as a positive control was consistently 18,000. The HP of E. coli strain Lilly R was 17,000. Hemagglutinating activity was detected with 6 of the 44 K. pneumoniae strains. Their HP values were: M13 - 2,400; M15 - 8; M19 - 32; N25 - 400; N26 - 2,700; and E33 - 130.

Serum and milk sensitivity tests

Figure 4 illustrates the CFU determination technique used for measurement of the bactericidal effect of serum or milk on the CFU count of K. pneumoniae strains. This procedure was chosen as being more accurate than the MAC agar plate procedure, since higher counts were obtained with tryptose agar, and a larger sample could be used (0.6 ml of each dilution compared to 0.15 ml on MAC agar). The tetrazolium in the tryptose agar was reduced and incorporated into the colonies. The resulting red color facilitated their identification and enumeration.

Initial sensitivity tests were carried out to determine the reproducibility of the system. K. pneumoniae strains M1, E32, B42, B43, and B44 were tested against various serum samples from different sows (Table 9).

CFU counts were not recorded at the 5-hour interval with the milk sensitivity test, since the number of bacteria always increased after 5 hours in milk. Strain M1 increased 16,000 CFU/ml after 2 hours in one milk sample, and increased 670,000 and 760,000 CFU/ml in another milk sample that was tested on two different occasions. When tested against

Figure 4. CFU determination technique utilizing disposo-trays and tryptose agar plus 0.005% triphenyl tetrazolium chloride.

Table 9. Reproducibility of serum sensitivity test with five strains of K. pneumoniae

Strain No.	Serum No.	CFU count at 0 hour	CFU count at 2 hours	CFU count at 5 hours	CFU change 0 to 2 hours	CFU change 2 to 5 hours
M1	3	86,000	1,200	640	-84,800	-560
	11	86,000	825	820	-85,175	-5
	12	74,000	1,200	91,000	-72,800	+89,800
E32	3	100,000	2,200	14,000	-97,800	+11,800
	11	100,000	1,700	3,000	-98,300	+1,300
	12	120,000	2,000	3,200	-118,000	+1,200
B42	12	160,000	2,900	25	-157,100	-2,875
	12	100,000	9,100	210	-90,900	-8,890
	17	130,000	17,000	380	-113,000	-16,620
B43	12	180,000	500,000	11,000,000	+320,000	+10,500,000
	15	98,000	470,000	26,000,000	+372,000	+25,530,000
	16	110,000	340,000	15,000,000	+230,000	+14,660,000
	17	140,000	480,000	15,000,000	+340,000	+14,520,000
B44	12	260,000	0	0	-260,000	0
	15	140,000	0	0	-140,000	0
	16	180,000	0	0	-180,000	0
	17	120,000	0	0	-120,000	0

these same milk samples, strain E32 decreased 150,000, 92,000, and 148,000 CFU/ml after 2 hours.

The 44 K. pneumoniae and 3 E. coli strains were tested for sensitivity to the bactericidal action of blood and milk. Mean values for the serum sensitivity of K. pneumoniae strains from three sources are presented in Table 10. The mean CFU/ml of strains from mastitic swine and swine environment decreased during the 0 to 2 hour test interval, and that of strains from normal swine increased. The difference was not significant, however, because of the great variability within each source. The milk sensitivity of strains from the three sources did differ significantly (Table 11). K. pneumoniae strains isolated from mastitic swine were significantly more resistant to the bactericidal action of milk than were strains from normal swine and swine environment.

Table 10. Comparison of serum sensitivity of K. pneumoniae strains from three sources

Source of strains	Mean CFU change		
	0 to 2 hours	2 to 5 hours	0 to 5 hours
Mastitic swine (n[a]=24)	-38,000	+1,185,000	+1,223,000
Normal swine (n=7)	+33,000	+5,179,000	+5,147,000
Swine environment (n=9)	-51,000	+42,000	+93,000
SD[b]	158,430	6,276,942	6,148,170
d.f.[c]	37	37	37

[a]n = number tested.

[b]SD = standard deviation.

[c]d.f. = degrees of freedom.

Table 11. Comparison of milk sensitivity of K. pneumoniae strains from three sources

Source of strains	Mean CFU change
	0 to 2 hours
Mastitic swine (n[a]=24)	+1,724,000
Normal swine (n=7)	+659,000
Swine environment (n=9)	+230,000
SD[b]	1,296,148
d.f.[c]	37
	**

[a]n=number tested.

[b]SD = standard deviation.

[c]d.f. = degrees of freedom.

**The difference between the mastitic swine mean and the normal swine and swine environment means is significant at P<0.01.

DISCUSSION

Comparison of two K. pneumoniae strains by inoculation into sow mammary glands indicated that one of swine mastitis origin (M1) was better able to establish and induced higher somatic cell counts than a strain isolated from the swine environment (E32). No difference was seen between the strains in the response of milk pH. The increase in mean pH values from 8 to 120 hours PI was suggestive of an inflammatory response to both strains, but the pH of milk from uninoculated control glands also tended to increase from 8 to 120 hours PI. The mean somatic cell counts, however, were consistently higher after 8 hours PI for the glands inoculated with strain M1 than for those inoculated with strain E32. Strain M1 also seemed to be better able to establish and grow in the sow mammary gland. The difference in CFU counts from glands inoculated with the two strains was evident until the 48-hour sampling. The assumption was made, at least until 48 hours PI, that the K. pneumoniae type reisolated from each gland was the same as that inoculated into it. There was no way to confirm this, however. At 48 hours PI, the numbers of K. pneumoniae isolated from all inoculated glands began to fluctuate, and the organisms could be isolated from the milk of uninoculated control glands. The reason for this spread of the organism was not immediately apparent. The piglets may have transferred the organisms from gland to gland while feeding. The increase in numbers shed by the sow may also have increased the population in the bedding, which could then reinfect the sow. These experiments were carried out in the summer months, when conditions of increased temperature and humidity would favor multiplication of Klebsiella in the wood shavings

(Thomas et al., 1960). It was noted that numbers of contaminating organisms isolated from milk samples increased greatly when the weather was most hot and humid.

Somatic cell counts in milk collected in this study appeared to be unusually high, even in the preinoculation samples. In a study of 314 milk samples from 84 sows, Schmid-Lindner (1965) recorded up to 2,000,000 cells/ml as the normal range, with counts up to 32,000,000 cells/ml and higher occurring in clinical cases of mastitis. In this study, a mean count of 15,868,187 cells/ml (SD 23,177,987) was obtained for 55 preinoculation samples. Counts from experimentally infected glands increased to as high as 287,700,000 cells/ml. The high somatic cell counts in preinoculation samples of milk may have resulted from constant exposure to contaminating bacteria from the environment.

The number of Klebsiella isolated from milk always tended to be higher from glands located to the rear of the udder than from the anterior glands. The opposite results might be expected, if there is some sort of local immunity to coliforms in the mammary gland, since this immunity would likely be stimulated by the frequent exposure to feces and contamination from the bedding. Berner and Marx (1967) reported that increased somatic cell counts occurred twice as often in rear glands as in anterior glands. They suggested that this increased cell count might be caused by trauma, since the glands were situated between the hind legs. However, the rear glands produce less milk than the anterior glands and are suckled less (Gill and Thomson, 1956), which might give the organisms more time and opportunity to establish. For this same reason, the number of pigs in a litter may have influenced the results obtained from a given sow.

Litters with small numbers of piglets might result in incomplete removal of milk from some glands, and bacteria would remain in such glands longer than in others.

Stress and hormone changes are important factors in decreased resistance to infection (Glynn, 1972; Shackelford and Feigin, 1973; Wagner, 1974). The hormone changes occurring at parturition are likely important factors in determining the incidence of mastitis cases that develop within 48 hours of farrowing. Stress resulting from moving the sow into farrowing crates shortly before parturition may also play a role in increased susceptibility to infection at this time. Very hot weather may not only promote survival and multiplication of potentially pathogenic bacteria, but may weaken the host response to the bacteria as well. To date, there have been no studies on the incidence of mastitis with respect to time of year or prevailing weather conditions. It is known that Klebsiella spp. are primarily pathogenic for humans whose immunologic defenses are lowered by disease or suppressive drugs, or whose bacterial flora has been altered by antibiotics (McCabe and Jackson, 1962; Finland, 1973).

There are many sources of Klebsiella spp. in the swine environment, and some or all strains may be potentially pathogenic for the parturient sow. Klebsiella are found in swine drinking water and on walls and floors (Nieva, 1971; Braman et al., 1973). The organisms were also isolated from swine feed as part of this project. Wood by-products, often used as bedding for the sow, have been incriminated as the source of mastitis-producing Klebsiella (Newman and Kowalski, 1973; Bramley, 1974). Bedding comes in close contact with the udder, and since the organisms survive and even multiply in wood by-products, perhaps this type of bedding should be

chemically treated or heated prior to use, or not used at all during this critical period.

Another source of infective Klebsiella spp. may be the feces of the sow, especially if conditions are such that there is extensive contact with the sow's udder when she lies down. Confinement rearing systems which utilize farrowing crates usually do not ensure adequate removal of feces. Murphy and Hanson (1943), when discussing feces as the possible source of mammary infection in cows, felt that the predominance of E. coli in the feces and not in the mammary gland might be indicative of the greater intramammary pathogenicity of Klebsiella spp. Salzman et al. (1967) found Klebsiella and Enterobacter spp. on hands of hospital personnel much more often than E. coli and suggested that these species must be more stable than E. coli. Thomas et al. (1960), however, pointed out that citrate-utilizing organisms outlive E. coli in any environment other than the intestine, and exposure to them is therefore greater. Bramley (1974) indicated that citrate positive coliforms survive longer than E. coli on a cow's udder skin as well as in sawdust. It is also possible that antibiotics used in swine feed increase intestinal carriage of Klebsiella, which in turn increases the number of Klebsiella infections. Intestinal carriage of Klebsiella in patients on antibiotic therapy has been proposed as a major cause of Klebsiella infections in hospitals (Selden et al., 1971).

The milk sensitivity test may have value as an in vitro test for determination of the pathogenicity of Klebsiella strains of swine origin. On the other hand, the serum sensitivity test appeared to have little value for this purpose, since it did not detect any significant differ-

ences among strains of K. pneumoniae isolated from mastitic swine, normal swine, or swine environment. Bactericidal activity was greater in serum than in milk; 33 of 44 strains were sensitive to the action of serum, while only 11 strains were sensitive to the action of milk. It is not known whether serum contains greater concentrations of the same bactericidal substances contained in milk, or whether the greater activity of serum is due to bactericidal systems not present in milk. Strains from mastitic swine were significantly more resistant to the bactericidal activity of milk than were strains from normal swine and the environment. K. pneumoniae strain M1, which was better able to establish in the sow mammary gland than strain E32, was also resistant to the in vitro bactericidal action of milk. Strain E32 was sensitive to this action. Both strains were sensitive to the bactericidal activity of serum.

It is difficult to correlate the milk or serum sensitivity of Klebsiella strains with any biochemical, morphologic, or serologic characteristics. The susceptibility or resistance of organisms to serum may be related to the state or amount of O antigen present. Serum-resistant strains were reported to be more potent sources of somatic antigens and endotoxin than serum-sensitive strains (Michael and Landy, 1961; Carroll et al., 1969). Howard and Glynn (1971) found that both O and K antigens were decreased in amount in serum-sensitive strains. Complement resistance was proportional to the amount of K antigen present, though various K antigens differed in their effectiveness. The authors felt that serum-susceptibility might be caused by the removal of the inhibitory effects of K antigen, or possibly by the exposure of hidden O antigenic determinants. Muschel (1960) pointed out that an organism's serum-

resistance is associated with its O-inagglutinability, which is believed to result from the presence of K antigens. Glynn (1972) indicated that K antigen and O-inagglutinability are related to increased resistance to both complement-killing and phagocytosis. The amount of K antigen present seems to be of importance. Complement-sensitive and resistant strains of the same E. coli K (and O) serotype are not uncommon. Also, K antigen has been shown to reduce the amount of both IgG and IgM antibody attachment.

All Klebsiella strains not K. ozaenae or K. rhinoscleromatis are considered to be K. pneumoniae according to the 8th edition of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974). This classification is widely accepted in the United States. The strains classified as K. pneumoniae according to this scheme are a heterogeneous group biochemically. In England, Cowan et al. (1960) have developed a biochemical classification of Klebsiella strains, naming five species and one variety. They are K. ozaenae, K. rhinoscleromatis, K. aerogenes, K. pneumoniae, K. edwardsii var. edwardsii, and K. edwardsii var. atlantae. The K. aerogenes, K. pneumoniae, and K. edwardsii of the Cowan classification are all included in the K. pneumoniae classification used in the United States. Foster and Bragg (1962) attempted to correlate the Cowan biochemical classification with the severity of chest infections caused by Klebsiella spp. They found that K. edwardsii var. edwardsii tended to be the most pathogenic and K. aerogenes the least. They concluded, however, that, although there is a wide variation in virulence between species, there appears to be no clear differentiation into pathogenic and saprophytic species with respect to man. Darrell and Hurdle (1964) and Fallon (1973), in similar investigations, concluded that K. aerogenes was most

commonly isolated from clinical specimens, but was of doubtful pathogenicity. This species was almost always isolated after antibiotic therapy, probably as a secondary invader after a previous pathogen. Of the 44 K. pneumoniae strains characterized biochemically in this study, 35 would be K. aerogenes according to the classification of Cowan et al.

Of these 44 K. pneumoniae strains, 4 (10%) were indole positive. The number of indole positive Klebsiella isolates seems to be increasing, with from 16% to 33% being reported in some studies (Rennie and Duncan, 1974; Davis and Matsen, 1974). Davis and Matsen (1974) suggested that this was due to the higher percentage of Klebsiella spp. isolated from stool specimens since the development of more selective media, such as citrate agar. They speculated that Klebsiella grow in close contact with many indole positive species of bacteria in the intestine and may acquire this characteristic from them.

Strains of K. pneumoniae isolated from mastitic swine were more resistant to penicillin according to the analysis presented in Table 7, and more resistant to streptomycin according to the analysis presented in Table 8, than were strains from normal swine or swine environment. Since a combination of penicillin and streptomycin is often used in swine rations, more strains resistant to these antibiotics might be expected from swine than from the environment. The reason for the greater resistance of strains from mastitic swine than from normal swine is not apparent, however. If there were a correlation between pathogenicity and resistance to antibiotics, it might be assumed that antibiotics in feed would suppress sensitive bacteria, thereby allowing resistant bacteria to increase in numbers and cause disease. However, there is no evidence that

antibiotic-resistant organisms are more pathogenic than antibiotic-sensitive organisms.

The hemagglutination of guinea pig erythrocytes is used as an indicator of the presence of surface appendages on certain bacteria, which are called fimbriae or pili. Fimbriae have been associated with virulence in various species of bacteria, presumably due to their ability to attach to host cells (Duguid and Gillies, 1957; Swanson, 1973; Sandhu et al., 1974) or to resist phagocytosis (Ofek et al., 1974).

Fimbriae are reported to be present on most strains of K. pneumoniae (Ørskov, 1974). Using the Cowan classification, Duguid (1959) suggested that K. aerogenes, which he considered to be saprophytic, was fimbriate, while the pathogenic K. pneumoniae, K. ozaenae, and K. rhinoscleromatis were not. He felt that fimbriae on an animal pathogen would hinder it by promoting attachment to phagocytic cells. Saprophytic strains, however, would be aided by their ability to attach to intestinal epithelium, root hairs, and the surface of stagnant water. Cowan et al. (1960), in their biochemical classification, reported that fimbriae were present on K. aerogenes and K. pneumoniae and absent on K. edwardsii and K. rhinoscleromatis.

Only six of the 44 K. pneumoniae strains (Bergey's classification) tested in this study had hemagglutinating activity: three were from mastitic swine, two from normal swine, and one from the environment.

Fimbriae can be visualized only by electron microscopy. Since this was not included in this study, it is possible that fimbriae were present on most strains, and the hemagglutination test failed to detect them. It

is also possible that they were of the mannose-resistant, thick type, which are best detected by hemagglutination of tanned ox erythrocytes. However, the majority of Klebsiella strains are reported to have the mannose-sensitive, thin type of fimbriae, which are best detected by the guinea pig erythrocyte hemagglutination test (Duguid, 1959).

SUMMARY

Comparisons were made of Klebsiella pneumoniae strains isolated from mastitic swine, normal swine, and their environment using animal inoculation, in vitro bactericidal tests, and certain other in vitro procedures. The objective was to determine whether strains of the organism isolated from diseased sows had characteristics that could be used to distinguish them from nonpathogenic strains.

In initial work, five sows were used to determine the optimal route, medium, and amount of inoculum to use for in vivo comparison of the strains. Five additional sows were then used to compare strains from the three sources by evaluating their establishment and the response of the animal after intramammary inoculation. A strain isolated from mastitic swine was better able to establish in the mammary gland and elicited higher somatic cell counts in milk than did a strain from the environment.

The K. pneumoniae strain of mastitis origin that was more virulent in vivo was also resistant to the bactericidal activity of milk in vitro. The strain from the swine environment that was somewhat less virulent in vivo was sensitive to the bactericidal activity of milk in vitro.

Comparison of 40 K. pneumoniae strains using the in vitro bactericidal tests indicated a correlation between milk sensitivity and pathogenicity, but no correlation between serum sensitivity and pathogenicity. Strains isolated from mastitic swine were significantly more resistant to the bactericidal activity of milk than were strains isolated from normal swine or the swine environment.

In additional *in vitro* comparisons, the majority of 40 strains from mastitic swine, normal swine, and their environment were of similar biotypes (33 of 40), differing only in fermentation of dulcitol. There was no difference in mean capsule size among strains from the three sources. The sensitivity of the strains to seven antibiotics varied, with those from mastitic swine being more resistant to ampicillin, penicillin, and streptomycin, and those from the environment being more sensitive to cephalothin. Six of the 40 strains agglutinated guinea pig erythrocytes.

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